#38
DmJ
7-

COPY OF PAPERS
ORIGINALLY FILED

MSU 4.1-406
06/10/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Alberto L. Mendoza

Serial No.: 09/082,112 Group Art Unit: 1647

Filed : 1998 May 20

For : METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

Examiner : Sharon L. Turner

Assistant Commissioner For Patents

Washington, D.C. 20231

BRIEF UNDER 37 C.F.R. § 1.192

Sir:

This is an appeal from a final rejection in the above entitled application. The claims on appeal are set forth as Appendix A. An oral hearing will be requested. Enclosed are three (3) copies of this Brief and the fee due upon filing of the Brief.

(1) Real Party in Interest

The real party in interest is the Board of Trustees operating Michigan State University, East Lansing, Michigan, a constitutional corporation of the State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

This application is a divisional of Application Ser. No. 08/895,940 ('940), which was filed July 17, 1997, and is now U.S. Patent No. 5,948,413. Another divisional of the '940, Application Ser. No. 08/082,232 ('232), was filed May 20, 1998. The '232, which relates to an injectable vaccine for Pythiosis, is under appeal. There are no related interferences.

(3) Status of Claims

Claims 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25 are pending. All claims are rejected. Claims 1-15 were canceled in a preliminary amendment which was filed May 20, 1998.

(4) Status of Amendments

An Amendment After Final filed May 3, 2002, was not entered.

(5) Summary of Invention

The invention provides a method for treating Pythiosis in humans (Claims 16 and 17) and mammals (Claims 18-25) using the vaccine prepared according to the method of Example 1 (Specification: page 6, line 34 to page 8, line 2).

In particular, the applicant provides a method for treatment of Pythiosis in human patients having the

Pythiosis (Specification: page 5, lines 13-18; Example 4, page 9) which comprises (a) providing a vaccine containing a mixture of mixed intracellular proteins and mixed extracellular proteins of *Pythium insidiosum* in a sterile aqueous solution (Specification: page 7, lines 1-16 and 28-35), wherein the mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* grown in a culture medium (Specification: page 7, lines 3-15), and the mixed extracellular proteins, which consist essentially of proteins removed from the culture medium for growing the *Pythium insidiosum* (Specification: page 7, lines 3-7), are in water (Specification: page 7, lines 32-33) and the mixture has been dialyzed to remove low molecular weight components less than 10,000 MW (Specification: page 7, lines 34-35); and (b) vaccinating the patient with the vaccine (Specification: page 5, lines 13-18).

In a preferred embodiment, the patient is vaccinated with the vaccine subcutaneously (Specification: page 10, lines 18-20; page 13, lines 34-36).

The applicant also provides a method for the treatment of Pythiosis in a mammal having the Pythiosis (Specification: Example 2, page 8, line 3, to page 7, line 19) which comprises (a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum*

in a culture medium (Specification: page 7, lines 1-16 and 28-35) which comprises in a sterile aqueous solution in admixture:

(1) mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* separated from the culture medium (Specification: page 7, lines 3-15); and

(2) mixed extracellular proteins, which consist essentially of proteins removed from the culture medium separated from the cells of the *Pythium insidiosum* (Specification: page 7, lines 3-7);

wherein the admixture in water (Specification: page 7, lines 14-16 and 32-33) has been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine (Specification: page 7, lines 34-35); and (b) vaccinating the mammal with the vaccine.

In a further embodiment of the method, the removed proteins in the admixture have been provided by growing cells of the *Pythium insidiosum* in the culture medium (Specification: page 7, lines 1-2), then killing the cells (Specification: page 7, lines 3-4), then separating the killed cells from the culture medium to produce a first supernatant to provide the mixed extracellular proteins of (a)(2) (Specification: page 7, lines 3-7) and then disrupting the killed cells in sterile water (Specification: page 7, lines 8-11) and removing the disrupted cells from the sterile water

containing the mixed intracellular proteins to provide the mixed intracellular proteins of (a)(1) in a second supernatant (Specification: page 7, lines 12-16), combining the first and second supernatants (Specification: page 7, lines 14-16), precipitating the proteins (Specification: page 7, lines 28-29), resuspending the precipitated proteins in sterile water (Specification: page 7, lines 29-30), and dialyzing the resuspended proteins in sterile water to remove the material less than 10,000 MW (Specification: page 7, lines 34-35).

In a further embodiment, the cells have been disrupted by sonication (Specification: page 7, lines 8-10).

In a further still embodiment, the *Pythium insidiosum* is deposited as ATCC 74446 (Specification: page 6, line 35).

In a further still embodiment, the culture medium is Sabouraud's dextrose broth (Specification: page 6, line 37).

In a further still embodiment, the cells are killed with thimersol (Specification: page 7, lines 3-4).

In a further still embodiment, the disrupted cells are removed from the sterile water containing the mixed intracellular proteins by centrifugation to provide the mixed intracellular proteins of (a)(1) in

the second supernatant (Specification: page 7, lines 12-13).

In a further still embodiment, the mixed intracellular and extracellular proteins from (a)(1) and (a)(2) are precipitated with acetone to produce a precipitate and resuspending the precipitate in sterile distilled water for the dialysis ((Specification: page 7, lines 28-33).

(6) Issues

(a) Claims 19 and 24 were rejected under 35 U.S.C. § 112, second paragraph.

In particular, the phrase "removing the disrupted cells from the sterile water containing the mixed intracellular proteins to provide the mixed intracellular proteins of (a)(1) in a second supernatant" was stated to be indefinite as to what is being removed or what steps are being performed.

(b) Claims 16-25 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Mendoza *et al.* (1996), Mendoza *et al.* (1992a) (IDS: AI), Mendoza *et al.* (1992b) (IDS: AJ), Sigma Catalogue (1992), Amicon Catalogue (1993), and Mendoza abstract (1995).

In particular, it is stated that because Mendoza (AI) teaches a first vaccine consisting solely of the disrupted cell mass of *Pythiosis insidiosum* (CMV) and a second vaccine consisting solely of proteins

secreted by *Pythiosis insidiosum* in cell culture (SCAV), Mendoza (1996) and (1995) teach adding three particular intracellular antigens to the SCAV, Mendoza (AJ) teaches the identification of the three particular antigens by SDS gel electrophoresis, and Sigma and Amicon teach stir cells with P-10 membranes for removing low molecular weight material, it would have been *prima facie* obvious for one of ordinary skill in the art to make the applicant's vaccine which consists of the soluble intracellular and extracellular proteins in which the low molecular weight material has been removed by dialysis.

(7) Grouping of Claims

Claims 16 and 17, which stand and fall together, are patentably distinct from Claims 18-25, which stand and fall together.

Claims 16 and 17 relate to a method for treating pythiosis in humans and Claims 18-25 relate to a method for treating pythiosis in a mammal. The claims drawn to treating a human are patentably distinct because while technically humans are mammals, culturally humans are considered distinct and treatments which would be considered appropriate for mammals are generally not considered to be necessarily appropriate in humans.

(8) Argument

(a) Claims 19 and 24 are not indefinite under 35 U.S.C. § 112, second paragraph.

The phrase "removing the disrupted cells to provide the mixed intracellular proteins" is believed to clearly inform a person of ordinary skill in the art of what is being removed.

As recited in Claims 19 and 24, the killed cells are disrupted in sterile water and the disrupted cells are then removed from the sterile water, which leaves the mixed intracellular proteins in the sterile water. A person of ordinary skill in the art would know that killed cell debris is insoluble in sterile water and that the most common method for removing the disrupted killed cells is centrifugation and that after centrifugation, the supernatant fraction would contain the mixed intracellular proteins which would be soluble. Thus, a person of ordinary skill in the art would not have any doubt as to what is being claimed.

Furthermore, to provide guidance to one of ordinary skill in the art, the applicant teaches in Example 1 disrupting the killed cells in sterile water by sonication and then removing the killed cells by centrifugation.

In light of the above, reversal of the rejection and remand to the Examiner for allowance is requested.

(b) The applicant's claimed method for treating Pythiosis in mammals (Claims 18-25) and humans (Claims 16 and 17) is not under 35 U.S.C. § 103(a) unpatentable over Mendoza *et al.* (1996), Mendoza *et al.* (1992a) (IDS: AI), Mendoza *et al.* (1992b) (IDS: AJ), Sigma Catalogue (1992), Amicon Catalogue (1993), and Mendoza abstract (1995).

The prior art would not have rendered the applicant's claimed invention *prima facie* obvious. M.P.E.P. § 706.02(j) sets forth the criteria that must shown to establish that a claimed invention is *prima facie* obvious in view of a combination of prior art references. To establish *prima facie* obviousness, it must be shown that (1) there is some suggestion or motivation, either in the prior art references or the general knowledge of one of ordinary skill in the art to combine the reference teachings, (2) there is a reasonable expectation of success if the teachings of the prior art references were combined, and (3) the combined prior art references must teach or suggest all of the claim limitations. It is particularly important to show that there is some reason why one of ordinary skill in the art, with no knowledge of the claimed invention, would have selected the particular prior art references and combined them to render the claimed invention obvious. The case law has repeatedly insisted on such a showing (*See* In re Sang Su Lee, 61 USPQ2d

1430, 1433 (Fed. Cir. 2002), for a brief review of the case law).

In the present case, the prior art provides no suggestion or motivation to one of ordinary skill in the art to combine the prior art references to produce and use a vaccine like the applicant's for treating mammals (Claims 18-25) or humans (Claims 16 and 17) infected with *Pythiosis insidiosum* with any expectation of success. Furthermore, even when the prior art references are combined, the combined prior art references do not teach or suggest every element of the applicant's claimed method.

The applicant's presently claimed method uses a vaccine that contains all the soluble intracellular proteins and the extracellular proteins in a mixture wherein material less than 10,000 MW has been removed. The applicant's vaccine has therapeutic characteristics that are more effective than either the CMV (intracellular protein vaccine) or SCAV (extracellular protein vaccine) of the prior art. Even though the applicant's vaccine contains intracellular proteins, unlike the CMV, the applicant's vaccine does not cause a prominent inflammatory response at the site of inoculation. The applicant's vaccine is able to cure horses that have been chronically infected with *P. insidiosum* for greater than 60 days (Specification: page 8, lines 22-27) and to cure all horses that had acute

cases of *P. insidiosum* (Specification: page 8, lines 32-33). Furthermore, the applicant also provides in Example 4, the remarkable ability of the claimed vaccine to cure a human who had been infected with *P. insidiosum* for over 60 days. None of the prior art even hints at that possibility.

Mendoza (1992a) teaches two methods for producing *Pythium insidiosum* vaccines, a cell-mass vaccine (CMV), which contains both soluble and insoluble proteins, and a soluble concentrated antigen vaccine (SCAV) which contains solely extracellular proteins. Both vaccines were of limited value for treating horses infected greater than 0.5 months but less than 2 months, and neither vaccine was effective for treating horse that had been infected for more than 2 months. Importantly, Mendoza (1992a) teaches that the CMV vaccine is undesirable because it has a short shelf-life and it causes a prominent inflammatory response at the site of inoculation. Furthermore, Mendoza (1992a) does not suggest that either the CMV or the SCAV would be able to cure Pythiosis in humans. Thus, in light of Mendoza (1992a) one of ordinary skill in the art would not have been motivated to make a vaccine containing soluble intracellular proteins to provide a method for curing Pythiosis in mammals (Claims 18-25) or humans (Claims 16 and 17).

Mendoza (1992b) teaches preparing a mixture of

intracellular proteins from *Pythiosis insidiosum* for use in Western blots. Mendoza (1992b) teaches disrupting the cells and then removing the disrupted cell debris by centrifugation to produce a supernatant containing the proteins. As a person of ordinary skill in the art knows, the only reason the disrupted cell debris was removed from the intracellular proteins was to prevent the disrupted cell debris from clogging up the gel well thereby preventing the intracellular antigens from entering the gel. Mendoza (1992b) does not suggest such a preparation has any use in a vaccine for treating Pythiosis in mammals (Claims 18-25) or humans (Claims 16 and 17). As far as one skilled in the art would know, the composition of Mendoza (1992b) would still have a short shelf-life and produce a prominent inflammatory response at the site of inoculation. Thus, one of ordinary skill in the art in view of Mendoza (1992a) and Mendoza (1992b) would not have been motivated to make a vaccine containing soluble intracellular proteins for treating Pythiosis in mammals (Claims 18-25) or humans (Claims 16 and 17).

Mendoza (1995) discloses an SCAV vaccine containing three immunodominant intracellular proteins and Mendoza (1996) discloses an SCAV vaccine containing "cytoplasmic antigens, including the 28K, 30K and 32K immunodominant proteins . . . ." Both vaccines were reported to be able to cure horses chronically infected

with *P. insidiosum*. Both Mendoza (1995) and Mendoza (1996) teach that the preferred vaccine would consist of the SCAV and the three immunodominant proteins. Neither suggests to a person of ordinary skill in the art to make a vaccine that contained all the soluble intracellular proteins (but not the insoluble proteins) and the extracellular proteins. At best, one of ordinary skill in the art would most likely be motivated to make a vaccine consisting of the SCAV and the three immunodominant proteins. But that vaccine would not contain all of the soluble intracellular proteins. Furthermore, neither Mendoza (1995) or Mendoza (1996) suggest that such a vaccine could be used to cure Pythiosis in a human as claimed in Claims 16 and 17.

Because of Mendoza (1992a), one skilled in the art would not have been motivated to make a vaccine that consisted of the SCAV and intracellular proteins. Even if one skilled in the art were motivated to make a vaccine that consisted of the SCAV and intracellular proteins as taught in Mendoza (1992a), the vaccine would contain the insoluble intracellular proteins which are not present in the applicant's vaccine. In addition, unlike the applicant's vaccine, both vaccines made in view of the prior art would contain material less than 10,000 MW. Thus, neither vaccine made in view of the prior art would have all the elements of the applicant's claimed vaccine, that is consisting of only soluble

intracellular proteins and extracellular proteins wherein the proteins have a molecular weight greater than 10,000 MW.

It would have been particularly unlikely that one skilled in the art would have made the applicant's vaccine because Mendoza (1992a) teaches the CMV is unstable and causes a prominent inflammatory reaction at the site of inoculation. Therefore, in light of that and Mendoza (1995) and Mendoza (1996), which teach combining the three immunodominant proteins to the SCAV, a person of ordinary skill in the art would not have been motivated to add all of the soluble intracellular proteins to the SCAV because in view of the prior art (Mendoza (1992a), the skilled artisan would have expected the vaccine to have a short shelf-life, limited efficacy, and cause a prominent inflammatory reaction at the site of inoculation. Even though Mendoza (1992b) teaches a composition containing soluble intracellular proteins but which further includes material less than 10,000 MW and insoluble proteins, there is nothing in Mendoza (1992b) or Mendoza (1992a) or Mendoza (1995) or Mendoza (1996) which would suggest to one of ordinary skill in the art that adding only the soluble intracellular proteins of the CMV to the SCAV would produce a vaccine with an efficacy superior to either prior art vaccine and a vaccine which would not have the undesirable attributes of the CMV.

Even when the prior art is considered further in view of Sigma and Amicon, the applicant's vaccine is not rendered *prima facie* obvious. Sigma and Amicon teach using stir cells to concentrate proteins in a solution under positive pressure. Stir cells concentrate proteins in a solution by forcing liquid containing the proteins through a membrane. A stir cell fitted with a membrane with a 10,000 MW cut-off will concentrate all material with a molecular weight greater than 10,000. However, the stir cell does not remove material less than 10,000 MW and replace it with water. The membrane merely prevents material greater than 10,000 MW from being lost as the volume of the filtrate is reduced during the process of forcing the filtrate through the membrane. Therefore, while the concentration of material greater than 10,000 MW in the filtrate increases as the volume of the filtrate is decreased, the concentration of material in the filtrate less than 10,000 MW remains the same. Thus, after concentrating the filtrate using a stir-cell, the filtrate still contains the same concentration of less than 10,000 MW material.

In contrast to concentrating a sample using a stir-cell, dialysis works under the principle of equilibrium to remove small molecules and exchange solvents. In dialysis, a sample such as the above filtrate is placed in bag consisting of a dialysis

membrane, which is then placed in a large volume of a solvent. The membrane allows small molecules to pass freely through the membrane while retaining larger molecules which cannot pass through the dialysis membrane. Dialysis membranes are available with different MW cut-offs. For example, the dialysis membrane used by the applicant allows only molecules less than 10,000 MW to pass through the dialysis membrane. Therefore, when the sample in the dialysis membrane is placed in a large volume of solvent that does not contain the small molecules in the sample, the small molecules diffuse from the sample into the solvent until equilibrium is reached wherein the concentration of small molecules in the solvent becomes the same as the concentration of small molecules in the sample. Thus, the concentration of small molecules in the sample is reduced relative to the concentration of large molecules. By changing the solvent each time after equilibrium is reached, the concentration of small molecules in the sample can be completely removed or reduced to a negligible level. The same principle enables the solvent of the sample to be exchanged with another solvent.

Except in the case where the large volume of solvent contains a high salt concentration, dialysis does not result in the sample becoming concentrated; dialysis merely changes the composition of the sample by

removing or introducing small molecules that can pass through the membrane. Therefore, a protein preparation containing low molecular weight material that is dialyzed results in a preparation that is distinguishable from the product that results when the same protein preparation is concentrated using a stir-cell because in the former the concentration of low molecular weight molecules is reduced or removed whereas in the latter the concentration of low molecular weight material remains the same.

Therefore, in light of the above, neither Sigma nor Amicon would have rendered the applicant's vaccine *prima facie* obvious. While Mendoza (1992a) teaches using an Amicon stir-cell fitted with a PM-10 filter (Sigma) to concentrate only the proteins in the SCAV, none of the prior art teaches using the Amicon stir cell fitted with the PM-10 filter to concentrate intracellular proteins or that the Amicon stir cell fitted with the PM-10 filter is used for removing material less than 10,000 MW. Even if the Amicon stir cell fitted with the PM-10 filter did remove some or all of the extracellular material less than 10,000 MW, combining the extracellular material concentrated with the Amicon stir cell fitted with the PM-10 filter with the intracellular material of Mendoza (1992b) would produce a composition that contained intracellular material less than 10,000 MW. That composition would be

distinguishable from the applicant's vaccine. There is simply nothing in the prior art that would suggest to one of ordinary skill in the art that combining the soluble intracellular proteins with the extracellular proteins and then removing material less than 10,000 MW would produce a vaccine with the ability to cure horses chronically infected with Pythiosis but which retained all the desirable properties of the SCAV, that is, did not have the undesirable attributes of the CMV (Specification: page 9, lines 8-14). Nor is there anything in the prior art that would have suggested that such a vaccine could cure a human chronically infected with Pythiosis (Claims 16 and 17). Therefore, the prior art simply does not render *prima facie* obvious the applicant's vaccine.

The only way for one of ordinary skill in the art to go from an SCAV which contains the three immunodominant proteins (Mendoza (1995) and Mendoza (1996)) to the applicant's vaccine in light of the prior art which teaches that a vaccine containing all the intracellular antigens is undesirable (Mendoza (1992a) is to improperly use the applicant's disclosure as prior art. Without the applicant's disclosure, there is no other way one of ordinary skill in the art would expect that an efficacious vaccine could be made by combining extracellular antigens with all the soluble intracellular antigens and removing material less than

10,000 MW. Nothing in the prior art suggests that preparing a vaccine against Pythiosis in the manner taught by the applicant would remove the undesirable attributes of the CMV without affecting the properties of the SCAV and which produce a vaccine with the ability to cure chronically infected horses similar to a vaccine consisting of the SCAV and the three immunodominant proteins. Nothing in the prior art would have suggested that such a vaccine could cure a human chronically infected with Pythiosis. The knowledge that the CMV could be modified as taught by the applicant, combined with the SCAV, and then dialyzed to remove material less than 10,000 MW to produce a vaccine with the ability to cure chronically infected horses and humans could only be gleaned from the applicant's disclosure. Thus, the present rejection is based upon hindsight reasoning.

Therefore, in light of the above, Claims 16-25 are not *prima facie* obvious over the prior art. Reversal of the rejection and remand to the Examiner for allowance is requested.

(9) Conclusion

For the above reasons, Claims 16 to 25, which relate to a method for treating *Pythiosis*-infected horses with applicant's vaccine, are patentable, and Claims 16 and 17, which relate to a method for treating *Pythiosis*-infected humans with applicant's vaccine, are

believed to be patentable. Reversal of the Examiner's rejections is requested and remand to the Examiner for allowance of Claims 16 to 25 is requested.

Respectfully,

Ian C. McLeod
Registration No. 20,931

McLeod & Moyne, P.C.
2190 Commons Parkway
Okemos, MI 48864

(517) 347-4100
Fax:(517) 347-4103

APPENDIX A

COPY OF PAPERS ORIGINALLY FILED

-16-

~~A method for~~ treatment of Pythiosis in human patients having the Pythiosis which comprises:

(a) providing a vaccine containing a mixture of mixed intracellular proteins and mixed extracellular proteins of *Pythium insidiosum* in a sterile aqueous solution, wherein the mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* grown in a culture medium, and the mixed extracellular proteins, which consist essentially of proteins removed from the culture medium for growing the *Pythium insidiosum*, are in water and the mixture has been dialyzed to remove low molecular weight components less than 10,000 MW; and

(b) vaccinating the patient with the vaccine.

-17-

The method of Claim 16 wherein vaccinating the patient with the vaccine is subcutaneous.

A method for the treatment of Pythiosis in a mammal having the Pythiosis which comprises:

(a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

(1) mixed intracellular proteins, which consist essentially of proteins removed from disrupted cells of the *Pythium insidiosum* separated from the culture medium; and

(2) mixed extracellular proteins, which consist essentially of proteins removed from the culture medium separated from the cells of the *Pythium insidiosum*;

wherein the admixture in water has been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine; and

(b) vaccinating the mammal with the vaccine.

-19-

The method of Claim 18 wherein the removed proteins in the admixture have been provided by growing cells of the *Pythium insidiosum* in the culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant to provide the mixed extracellular proteins of (a)(2) and then disrupting the killed cells in sterile water and removing the disrupted cells from the sterile water containing the mixed intracellular proteins to provide the mixed intracellular proteins of (a)(1) in a second supernatant, combining the first and second supernatants, precipitating the proteins, resuspending the precipitated proteins in sterile water, and dialyzing the resuspended proteins in sterile water to remove the material less than 10,000 MW.

-20-

The method of Claim 18 wherein the cells have been disrupted by sonication.

-21-

The method of Claim 18 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

-22-

The method of any one of Claims 19, 20 or 21 wherein the culture medium is Sabouraud's dextrose broth.

-23-

The method of Claim 19 wherein the cells are killed with thimersol.

-24-

The method of Claim 19 wherein the disrupted cells are removed from the sterile water containing the mixed intracellular proteins by centrifugation to provide the mixed intracellular proteins of (a)(1) in the second supernatant.

-25-

The method of Claim 19 wherein the mixed intracellular and extracellular proteins from (a)(1) and (a)(2) are precipitated with acetone to produce a precipitate and resuspending the precipitate in sterile distilled water for the dialysis.